

Advances in the study of endodontic infections: introduction

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The crown of this tooth was nearly all decayed, while the roots consisted of two branches, so that the very roots were uncommonly hollow and the holes in them stuffed with a soft matter. I took this stuff out of the hollows in the roots and mixed it with clean rain water, and set it before the magnifying glass so as to see if there were as many living creatures in it as I had aforetime discovered; and I must confess that the whole stuff seemed to me to be alive. . .

—Antonin van Leeuwenhoek

In this volume of *Endodontic Topics* conditions that allow microbes to invade and establish themselves in the root canal system of teeth and subsequently initiate as well as sustain apical periodontitis are reviewed. Proper understanding of the mechanisms involved is certainly critical to improve current as well as identify new clinical strategies for the combat of endodontic infections. As much of the available knowledge in endodontic microbiology derives from classic sampling, laboratory processing and phenotypic identification of root canal bacteria, preference has been given to advances, which have a basis in this methodology.

Although now and then put in doubt, a substantial body of research has provided evidence for the key role of microbial infection in the pathogenesis of apical periodontitis associated with diseased dental pulps. Already, van Leeuwenhoek in the 17th century made note of the presence of microorganisms in the root canal of a severely decayed tooth. However, it took over 200 years before his observation was confirmed and a cause and effect relationship was suggested by Miller (1). Two lines of experimental research in particular have contributed to our present understanding of the etiology of apical periodontitis; for the first the germ-rat studies carried out by Kakehashi et al. (2), and for the second, the experimental series conducted in the monkey involving microbial challenges of devitalized healthy pulps (3, 4). Clinical studies comparing the

microbial status of pulps in teeth suffering an ischemic injury from trauma and with post trauma radiographic bone lesion have provided yet another important evidence for bacterial infection as a crucial factor (5, 6). Sundqvist's thesis in 1976 (6), furthermore, became a cornerstone in the history of endodontic microbiology in the sense that it, by a detailed taxonomy, finally settled the significance of anaerobic bacteria.

The nature of the micro-biota in infected root canals has been the focus of considerable research over the years. An important objective of such studies has been to identify organisms or groups of organisms that are prevalent and, which may be linked to periapical lesion development and, therefore, are potential targets for therapeutic measures. Advancement of improved methodologies for both sampling and laboratory processing has been crucial to the achievements in this field of endodontontology. While many different morphotypes can be identified in smears (7) or by scanning electron microscopic observations of the root canal interior (e.g. (8)) viz. cocci, rods, filaments and spirochetes, it is by their multiplication in pure cultures their properties can be studied in greater detail and their pathogenic potential assessed.

Vital to the study of the involvement of bacterial organisms in various clinical presentations of apical periodontitis, whether silent or symptomatic, are properly taken and processed root canal samples.

Indeed much controversy and misleading interpretation, currently and in the past, have been caused by the lack of due attention to the risk of including irrelevant organisms from the oral environment in the sample. Many early studies also presented data based on unsuitable growth conditions, which resulted in gross underestimation of the contribution of the anaerobic segment of the root canal micro-biota. In this context the work of Möller (9) represents another milestone in the development of endodontic microbiology. In his thesis Möller targeted the fundamentals of root canal sampling. He then demonstrated the significance of proper isolation, disinfection and control of the operation field to avoid false positives. He further designed culture techniques to maximize the recovery of anaerobes and bacteria in small numbers under stress of root canal medicaments in treatment cases. A medium for transport (VMGA III) that would not allow growth but preserve viability of the sampled organisms, until being processed in the laboratory, was another significant addition to the state-of-the art culturing technique in endodontics.

It is obvious from direct observations of smears and the use of molecular techniques, including polymerase chain reaction that a significant portion of the micro-biota in infected, necrotic pulps fails to become identified by classic sampling, and traditional laboratory techniques. An important reason is that prevailing culturing methods, including culture media, often fail to satisfy the fastidious character of the bacterial organisms being present. Microorganisms in root canals may also be overlooked simply because they are outnumbered by overgrowth of other organisms in the sample or by dilution procedures. In the most recent years genotypic identification has been applied and used as a tool for re-classification of the root-canal flora (10). However, genotyping by amplifying genetic material directly sampled from infected root canals has yet to contend with the difficulty of deciding whether it is representative of the living organisms. Indeed, large portions of the organisms in biofilms, which are likely to exist on the inner walls of teeth with non-vital pulps, are not alive. Consequently, such organisms cannot be active producers of inflammatory substances of significance for lesion development and persistence. Yet, the introduction of molecular biology techniques to identify putative pathogens in endodontic infections has opened up new exciting perspectives and is likely to further our understanding

of the complex host-tissue parasite interactions in apical periodontitis. In this volume of Endodontic Topics the paper by Dr David Spratt of Eastman Dental Institute in London gives a detailed account and assesses pros and cons with various molecular techniques for the identification of root canal bacteria.

According to Dorland (11) infection can be defined as 'invasion and multiplication of microorganisms in body tissues, which may be clinically inapparent or result in local cellular injury because of competitive metabolism, toxins, intracellular replication, or antigen-antibody response'. Microorganisms present in root canals fulfill these criteria for infection; firstly, by having invaded a normally sterile tissue compartment, secondly, by causing a tissue reaction from their release of products during multiplication. It is reasonable to assume that the extent of bacterial exposure in apical periodontitis is related to the continued growth and metabolic activity of the organisms. In other words microorganisms must be viable to cause an infection. Consequently, factors that enable microorganisms to survive (persist) and grow should be regarded as virulence factors. Much of our knowledge on bacterial virulence in endodontic infections is related to the ability of bacteria to cause acute clinical symptoms including pain, tenderness to percussion, swelling and abscess formation as well as sinus tract. The results of many studies have indicated that only certain species of the root-canal flora are able to cause such signs and symptoms. While there is limited knowledge as to the key virulence factors in endodontic infections, the paper by Dr Ingar Olsen and Dr Gunnar Dahlén of the Universities of Oslo and Göteborg brings a review of the research on virulence factors such as capsule, toxins, enzymes factors for invasion, adhesion and interaction with host responses as they pertain to some bacteria implicated in acute endodontic infections viz. *Porphyromonas*, *Prevotella*, *Fusobacterium* and *Peptostreptococcus* spp.

The significance of anaerobic bacteria especially Gram-negative rods in acute lesions of apical periodontitis including abscess formation has been further pursued in a number of experimental studies in laboratory animals (see review by Dahlén (12)). It is apparent from these studies that bacterial interaction and cooperation is of outmost importance for bacterial survival, persistence and growth in the microbial community existing in root canals (13). Organization in biofilms may thereby be an important means. Yet,

the extent to which biofilms are established in endodontic infections has not been well documented. As settlement in biofilms is a common phenomenon whenever organisms are free-floating in aqueous environments, biofilm formation on the root canals walls in endodontic infections ought to be prevalent as well. If so, this would be an issue of substantial clinical significance as organisms residing in biofilms are known to better resist external adverse influences including antimicrobial treatment measures than organisms dwelling in a planktonic state. The paper by Dr Gunnar Svensäter and Dr Gunnar Bergenholz of the Universities in Malmö and Göteborg reviews the biofilm concept and how it may apply to endodontic infections. It is known that adhesion of microorganisms to surfaces triggers altered expressions of a large number of genes, which result in phenotypical changes. These genes may be transferred and shared by different species in a biofilm community and may provide important survival properties to the recipient organism. In this context bacterial plasmids are of great significance as they participate in the transfer of DNA. The paper by Dr Christine Sedgley and Dr Don Clewell of the University of Michigan details how DNA is exchanged between bacteria by means of plasmids. They further outline the significance and potential role of plasmids in the oral and the endodontic micro-flora.

As one would expect from the discovery of a dominance of anaerobes in primary endodontic infections (5, 6, 14, 15), the field of endodontic microbiology has devoted considerable effort to study this segment of the root canal micro-biota and especially so of its role in acute infections. It appears, however, that certain organisms viz. Gram-positive facultatives including enterococci (16–18), which often have a suppressed representation in primary endodontic infections, have a propensity to better resist antimicrobial endodontic treatment measures than anaerobes. This has shifted the focus somewhat in recent years to explore the microbiology of endodontically treated teeth and especially those with persistent signs of apical periodontitis (19–24), see also the review by Haapasalo et al. (25). It is clear from the literature that there is no standard procedure in terms of mechanical and antimicrobial measures that can predictably eliminate the infection in total. Especially organisms lodging in non-instrumented fins and crevices of the root canal system and in the dentinal tubules constitute a distinct challenge. The article by Dr Robert Love of the

University of Otago reviews current knowledge on the microbiology of dentinal tubule infections and the factors that may influence root canal bacteria to invade dentine. Dr Tuomas Waltimo, Dr Markus Haapasalo, Dr Matthias Zehnder and Dr Jürg Meyer of the Universities of Basel, British Columbia and Zürich have provided a review of the role and treatment of yeasts in endodontic infections. Dr Luis Chávez de Paz of Göteborg University, finally, relates to different aspects of Gram-positive organisms and their potential adaptive responses on being exposed to scarce nutritional supply and stress by endodontic treatments measures.

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